433.e1 COMMINS ET AL J ALLERGY CLIN IMMUNOL FEBRUARY 2009

TABLE E1. Details of UVa patient screening

UVa cases	No. of patients	No. of sera positive for IgE antibodies to $\alpha$ -gal >1.0 IU/mL	No. of sera positive for IgE antibodies to α-gal <1.0 IU/mL*
CIU cohort	28	0	3
CIU cohort controls	25	0	0
Angioedema	17	3	5
Anaphylaxis	19	6	1
Urticaria	51	6	5
Asthma	27	0	3
Atopic dermatitis	10	0	2
Chronic sinusitis	11	0	1
Allergic rhinitis	14	0	1
Other†	41	0	0
Total	243	15	21

 $\emph{UVa},$  University of Virginia;  $\emph{CIU},$  chronic idiopathic urticaria.

<sup>\*</sup>These 21 sera positive for IgE antibodies to  $\alpha$ -gal all have titers of less than 1.0 IU/mL, and these patients were not enrolled in the study and were not counted among the 24 cases.

<sup>†&</sup>quot;Other" includes nonallergic, oral allergy syndrome, eosinophilic esophagitis, allergic bronchopulmonary aspergillosis/mycosis, rash, and hypogammaglobulinemia.

**TABLE E2.** Inhibition RIA for  $\alpha$ -gal and Bradford protein assay of extracts

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	Dilution for 30% inhibition	Percentage inhibition at highest concentration	Protein concentration (mg/mL)
Beef			
Fresh	0.15*	78*	684
Commercial	0.51	54	693
Pork			
Fresh	0.27*	63*	884
Commercial	0.37	54	632
Lamb			
Fresh	0.3	59	563
Commercial	0.38	55	553
Chicken			
Fresh	NA	18†	775
Commercial	NA	27†	605

Inhibition RIA for  $\alpha$ -gal in fresh extracts of beef, pork, lamb, and chicken compared with commercial reagents. Values are expressed as the dilution of the respective extract to achieve 30% inhibition and the percentage of inhibition at the highest concentration of extract. The results represent the mean of 3 experiments performed in duplicate. A lower value is consistent with an increased presence of  $\alpha$ -gal. Briefly, galactose- $\alpha$ -1,3-galactose- $\beta$ -1,4-GlcNAc\*BSA was radiolabeled with iodine 125 by using the chloramine T technique. Serum negative for IgE antibodies to  $\alpha$ -gal with a high titer of IgG antibody was incubated for 3 hours with serial dilutions of pork thyroglobulin to construct a standard curve or serial dilutions of meat extracts. Radiolabeled  $\alpha$ -gal was then added to these dilutions and incubated for 3 hours. Goat anti-human IgG was then added for overnight precipitation. The following morning, samples were washed 3 times, and the amount of radiolabeled  $\alpha$ -gal in the precipitate was quantitated. *NA*, Dose for 30% inhibition is beyond the limit of detection for this assay.

Protein concentration was compared by using the Bradford method according to the manufacturer's specifications.

<sup>\*</sup>P < .01 for fresh extract compared with commercial reagent.

 $<sup>\</sup>dagger P < .01$  for the concentration of  $\alpha\text{-gal}$  in mammalian meat extracts compared with chicken.